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Age-Specific Prevalence and Transmission of TT Virus

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TT virus (TTV) is an unenveloped, single-stranded DNA virus that was discovered recently in the sera of Japanese patients with posttransfusion hepatitis of unknown etiology. A high prevalence of TTV infection in blood donors of several countries, including Brazil, has been demonstrated. To study the variation in TTV prevalence between different age groups, sera from 223 individuals without liver disease, aged 0–80 years, were tested by the polymerase chain reaction for the presence of TTV DNA. All subjects were inhabitants of the city of Rio de Janeiro, Brazil. The prevalence increased continuously with age ($P < .001$), from 17% among children under the age of 11 years, to 57% in people older than 50 years. To assess vertical transmission, sera from 105 unselected, consecutive parturient women attending a public maternity hospital were paired with cord bloods and examined for the presence of TTV DNA. Thirty-seven (35%) mothers were found to be TTV infected. Seven cord bloods were also positive, suggesting the possible transplacental transmission of the virus. Furthermore, a direct correlation between TTV viremia and presence of antibodies to the enterically transmissible hepatitis A virus (HAV) was observed in this group of women, with a relative risk of TTV infection of 5.09 (95% confidence interval 0.76–34.03) for women with anti-HAV, compared with women without. This finding suggested that the fecal-oral route might be an important route of TTV transmission. ***J. Med. Virol. 59:318–322, 1999.***

KEY WORDS: parvoviridae; liver disease; vertical transmission; fecal-oral route; Brazil

INTRODUCTION

Screening tests have been introduced into blood banks to avoid the transmission of hepatitis agents, such as hepatitis B virus (HBV) and hepatitis C virus. Despite these measures, sporadic cases of posttransfusion hepatitis are still observed among the recipients of blood units. Extensive efforts to identify new agents responsible for these cases have resulted in the discovery of parenterally transmissible viruses. One of these, named GB virus C (GBV-C) [Simons et al., 1995] or hepatitis G virus (HGV) [Linnen et al., 1996] is a member of the *Flaviviridae* family and can be transmitted vertically and sexually. GBV-C/HGV has a worldwide distribution. In developed countries, around 1–4% of volunteer blood donors are GBV-C/HGV viraemic, and a higher proportion has been found in various developing countries [Lampe et al., 1998a]. Although the prevalence of infection is greater among hepatitis patients than among healthy individuals, an association of GBV-C/HGV with liver disease remains unresolved. It is not known whether GBV-C/HGV is associated with other diseases in humans, is a passenger virus, or only becomes virulent under certain conditions [Mphahlele et al., 1998].

Another agent, TT virus (TTV), was first identified in a Japanese patient with posttransfusion non A-E hepatitis and named after the initials of this patient [Nishizawa et al., 1997]. TTV is an unenveloped, high-density virus, sharing some characteristics with parvoviruses. It has a single-stranded DNA genome of at least 3,739 bases [Okamoto et al., 1998b]. TTV infection is not restricted to hepatitis patients but is common in individuals without liver disease. In developed countries, prevalence in healthy populations have been reported that vary from 1–2% in North America [Charlton et al., 1998] and Scotland [Simmonds et al., 1998] to 10–13% in Germany [Viazov et al., 1998], England [Naoumov et al., 1998], and Japan [Okamoto et al., 1998b]. In rural populations of Asian, African, and South American developing countries, the rate of infected individuals varies greatly, from 7% in Sudan to 83% in The Gambia [Prescott and Simmonds, 1998].

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Such high frequencies suggest that TTV infection may persist for a long period of time and that a transmission route other than parenteral is likely.

Recently, a high TTV DNA seroprevalence was reported in Brazilian blood donors [Niel et al., 1999]. We now describe the age-specific prevalence of TTV infection in individuals without liver disease. The transplacental route of transmission is investigated by TTV DNA detection in paired serum samples from parturient women and umbilical cords. The possibility that TTV is transmitted via the fecal-oral route is also examined.

MATERIALS AND METHODS

Population Studied

The age-specific prevalence of TTV DNA was determined in blood samples collected during 1997–1998 from 223 individuals (129 male, 94 female) aged 0–80 years, without liver disease, and living in the city of Rio de Janeiro, Brazil. This group comprised 50 children and adolescents (0–17 years) whose blood was collected after diagnosis of exanthematous diseases, pneumonia, anemia, obesity, or toxoplasmosis, and 173 adults including 118 blood donors, 28 patients with diabetes, 8 with hypertension, and 19 whose blood was collected for routine analysis.

Another group of 105 unselected, consecutive pregnant women aged 14–40 years (mean ± SD, 24.7 ± 6.0 years), attending a public maternity hospital, was enrolled in a study of vertical transmission of TTV. As three women delivered twins, 108 children were born during the study. Paired blood samples were collected from mothers and umbilical cords.

Serology

Sera were tested for the presence of hepatitis B surface antigen (HBsAg) and antibodies to the hepatitis B core antigen (anti-HBc) using Hepanostika HBsAg Uni-form II and Hepanostika anti-HBc Uniform, respectively, in a microELISA system (Organon Teknika, Boxtel, The Netherlands) according to manufacturer's instructions. Anti-hepatitis A virus (anti-HAV) and anti-hepatitis E virus (anti-HEV) antibodies were detected using standardized "in-house" [Vitral et al., 1991] and commercial (HEV EIA kit, Abbott, Chicago, IL) enzyme immunoassays, respectively.

DNA Extraction and Polymerase Chain Reaction (PCR)

Total DNA was purified from 200 μl serum using the QIAamp blood kit (Qiagen, Hilden, Germany) and eluted in a final volume of 50 μl. Five microliters were added to a first round PCR performed for 35 cycles at 94°C, 1 min; 50°C, 1 min; 72°C, 2 min (and an additional 7 min at 72°C in the last cycle) in a final volume of 50 μl. One microliter of the first round PCR product was submitted to semi-nested PCR. This process was carried out for 35 cycles under the same conditions (only increasing the annealing temperature to 55°C). The PCR primers used were NG059, NG061, and NG063 [Okamoto et al., 1998b], modified as follows: external sense (first round) 5′ ACAGACAGRGGMGRAGGNAAYATG 3′, nt 1900–1923; internal sense (second round) 5′ GGNAAYATGYTRTGGATAGACTGG 3′, nt 1915–1938; antisense (both rounds) 5′ CTGGCATYTTWCCRTTTCCAAARTT 3′, nt 2185–2161. In the cases where TTV DNA was detected in only one member of a mother-cord serum pair, another assay was carried out using primers and PCR conditions previously described [Tanaka et al., 1998]. For some samples, this PCR assay was more sensitive. Seven microliters of each amplification product were electrophoresed in a 1.8% agarose gel. DNA was stained with ethidium bromide and visualized under ultraviolet light.

Statistical Analysis

Contingency table analysis (Chi-squared test for trend) was carried out to demonstrate the increase of TTV seroprevalence with age. Fisher's exact test was used to assess association between TTV viremia and anti-HAV seropositivity.

RESULTS

Age-Specific Prevalence of TTV Infection

A total of 223 sera from individuals aged 0–80 years and without liver disease were tested for the presence of TTV DNA. This testing was done by semi-nested PCR using primers from open reading frame 1. Ninety-two sera were positive, showing an overall prevalence of 41.3%. No significant difference was observed between infection rates among male (prevalence of 41.1%) and female (41.5%) individuals. The population was divided into age groups, with 35–40 individuals in each group. Figure 1 shows the curve of age-specific prevalence. The frequency of TTV infection increased gradually with age ($P < .001$), from 17% among children under the age of 11 years, to 57% in adults older than 50 years. The mean ages of the 92 positive and 131 negative individuals were 37.1 and 27.9 years, respectively. In people older than 20 years, the rate of TTV infection was 48%. Two children (of five tested) under 1 year of age were found to be TTV infected. This finding suggested the possibility that the TTV was transmitted by the mother-to-infant route.

Vertical Transmission

To test the hypothesis of vertical transmission, the presence of TTV DNA was sought in the sera of parturient women and infants at birth (cord blood). A group of 105 unselected, consecutive parturient women attending a public maternity hospital was enrolled in this study. The group was representative of the population of pregnant women living in the city of Rio de Janeiro. Mothers were 14–40 years old (mean age 24.7 years). Eight (8%) were anti-HBc positive, and two of these were HBsAg positive, anti-HBc IgM negative, that is, HBV chronic carriers. Three women delivered

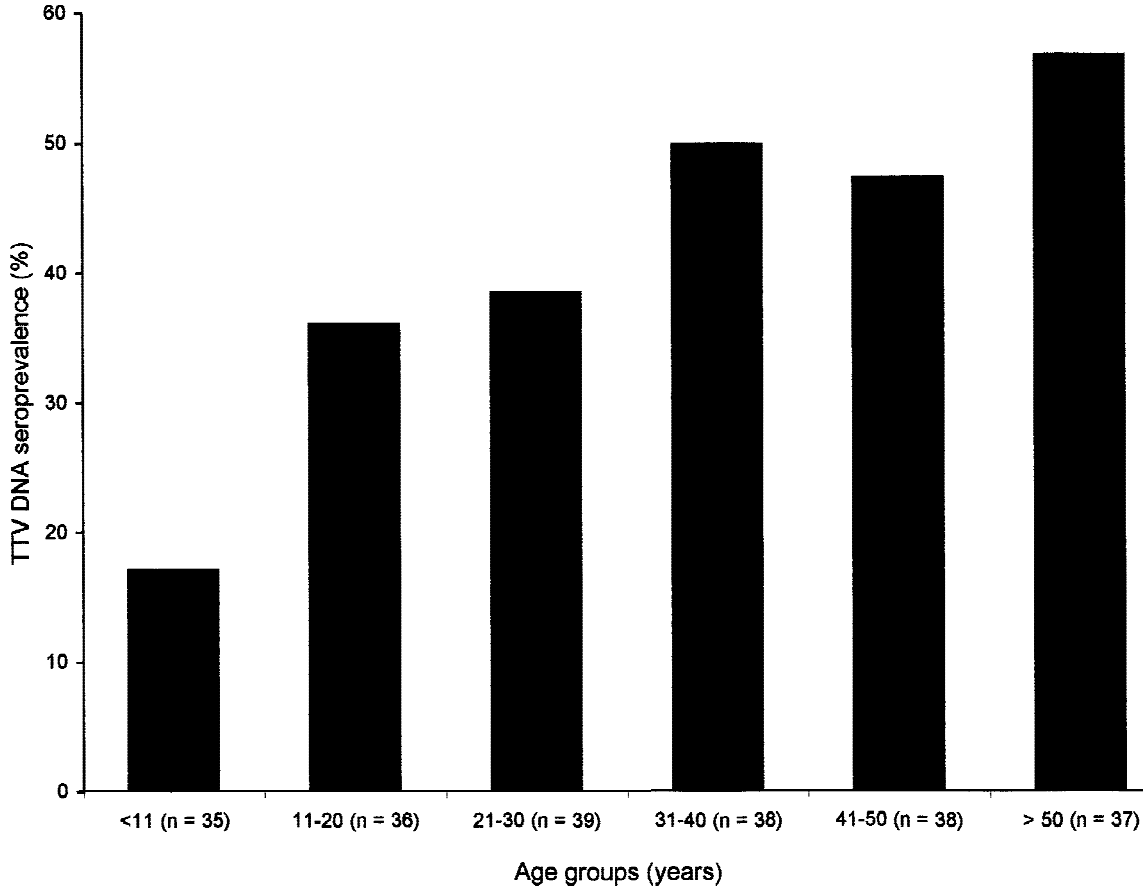

Fig. 1. Age-specific prevalence of serum TT virus (TTV) DNA in individuals without liver disease.

twins. Table I shows the detection of TTV DNA in paired sera from mothers and cords. A total of 37 (35%) sera from mothers were TTV DNA positive and 68 (65%) were negative. Seven (19%) cord bloods from the 37 positive mothers were also positive. All cords from TTV-negative mothers were also tested and all were TTV negative. The serum of one of the three mothers who delivered twins was TTV DNA positive, along with one of the related cords. However, TTV DNA was not detected in the serum of the other cord. The overall prevalence of TTV in cord bloods was 6.5%.

TABLE I. Detection of TT Virus DNA in Paired Sera of Mothers and Cords

Mother	Cord blood	Number of cases
One child		
Positive	Positive	6
Positive	Negative	30
Negative	Positive	0
Negative	Negative	66
Twins		
Positive	Positive (1), negative (1)	1
Negative	Negative (2)	2

Anti-HAV Seropositivity and TTV Infection

The curve of age-specific prevalence (Fig. 1), in conjunction with the results of vertical transmission (Table I), indicated that only a minority of TTV carriers have been infected perinatally. Together, mother-to-infant and parenteral transmission would not account for the high prevalence of TTV-infected adults. It has been suggested recently that TTV might be transmitted via the fecal-oral route [Okamoto et al., 1998a]. We therefore decided to seek an association between TTV infection and exposure to the enterically transmissible virus HAV. Table II shows the association between TTV viremia and anti-HAV seropositivity in the population of parturient women (cross-sectional study). Among the 37 TTV-infected mothers, 97% (95% confidence interval [CI] 92–100%) were anti-HAV positive, whereas among the 68 TTV-negative mothers, 82% (95% CI 73–92%) were anti-HAV positive. This difference in frequency was statistically significant ($P < .05$). The relative risk of TTV infection was 5.09 (95% CI 0.76–34.03) for women with anti-HAV as opposed to women without. A similar study was carried out with an anti-HEV assay, to correlate anti-HEV seropositivity and TTV carrier status of the mothers. However,

TABLE II. TTV Viremia and Anti-HAV Seropositivity in Parturient Women

	TTV		
Anti-HAV	Positive	Negative	Total
Positive	36 (97%)	56 (82%)	92 (88%)
Negative	1 (3%)	12 (18%)	13 (12%)
Total	37 (100%)	68 (100%)	105 (100%)

TTV, TT virus; HAV, hepatitis A virus.

only one woman was anti-HEV positive (not shown) and no association could be established.

DISCUSSION

TTV was first detected in the sera of three patients with posttransfusion hepatitis. TTV DNA titers correlated closely with aminotransferase levels in these patients [Nishizawa et al., 1997]. Also, TTV DNA has been found in liver tissues in titers equal to or higher than those in the corresponding sera [Okamoto et al., 1998b]. Therefore, the virus has been suggested as a causative agent of liver disease. However, the high prevalence of TTV infection in different population groups, including blood donors from different countries [Naoumov et al., 1998; Okamoto et al., 1998b; Prescott and Simmonds, 1998; Tanaka et al., 1998], raises the possibility that this virus is not pathogenic to humans [Viazov et al., 1998].

A high (62%) prevalence of TTV infection in blood donors living in the city of Rio de Janeiro, Brazil, has been demonstrated [Niel et al., 1999]. The occurrence of a high (41%) rate of infection in the general population, particularly in adults (48%), is confirmed by the present study. In a group of unselected parturient women (mean age 24.7 years), the frequency of TTV viremia was 35%. In Scotland, where the prevalence in blood donors is low (1.9%), TTV DNA has been found in the sera of people aged 30–67 years old (mean age 53 years) but not in younger blood donors [Simmonds et al., 1998]. This finding is consistent with our results showing a constant and gradual increase of TTV infection rates with age (Fig. 1). This pattern revealed a great divergence with the curve of age-specific prevalence of GBV-C/HGV, for which a maximum is reached in young adults (21–30 years) followed by a decline in older age groups [Lampe et al., 1998b]. In the case of TTV, the continuous increase of the curve suggests that the virus may not be eliminated, although the natural history of infection will remain conjectural until antibody tests are developed to show past infection and immunity [Cossart, 1998].

TTV was first characterized as a blood-borne virus and has thus been referred to as a "transfusion-transmitted virus" [Naoumov et al., 1998; Prescott and Simmonds, 1998; Simmonds et al., 1998]. However, high prevalence rates as mentioned above implicate the existence of other routes of transmission. Studies with paired serum samples from mothers and cords suggest that transplacental transmission of the virus might occur. However, the presence of TTV DNA in cord sera is not sufficient to prove the occurrence of transplacental transmission, due to possible contamination with blood of mother. Sequencing TTV DNA samples from paired cord serum and babies for the identical strain would be necessary for this purpose. Only a minority (19%) of cord bloods from the TTV-positive mothers was also positive. This low proportion may be due to the low viral load in blood. Indeed, a recent study [Simmonds et al., 1998] showed that TTV viremia ranged from 50 to 50,000 DNA copies/ml with a geometric mean of 620. In the course of this work, viral load was evaluated in some samples. No value higher than 50,000 DNA copies/ml was found (not shown). Our data showing a TTV DNA seroprevalence of 6.5% at birth, and a prevalence of 17% in children under the age of 11 (Fig. 1), indicate that many children are infected during the first years of life.

The shedding of TTV into feces from infected persons has been documented recently, suggesting the existence of a fecal-oral transmission route [Okamoto et al., 1998a]. Hepatitis A is an infection transmitted by the fecal-oral route. Endemicity within a specific country is directly related to sanitation and hygienic standards, while being inversely related to socioeconomic conditions. In our population of parturient women, the overall seroprevalence of anti-HAV was very high (88%). However, a significant difference in the rate of TTV infection could be noted between the groups of anti-HAV-positive and -negative women, the first group showing a higher seroprevalence of TTV DNA. This finding supports the fecal-oral transmission of TTV, although further studies involving a larger number of people, and populations of different origins, should confirm this point.

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